Attorney's Docket No. 5470-238 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: MacDonald et al.
Serial No.: 09/288,837
Filed: April 8, 1999
For: *METHODS AND MODIFIED CELLS* FOR THE TREATMENT OF CANCER

Art Unit: 1648
Examiner: Z. Lucas

December 12, 2003

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Dr. Ian Caley under 37 C.F.R. § 1.132

I, Ian Caley, do hereby declare and state as follows:

1. I am a business and licensing associate at AlphaVax, Inc., the exclusive licensee of United States Application Serial No. 09/288,837 (*hereinafter*, "the '837 application").

2. I received my undergraduate degree in Applied Biology, specializing in Microbiology at the University of Bath, England. I did my doctoral work in the laboratory of Dr. Robert Johnston (a named inventor on the '837 application), in the Microbiology and Immunology Department at the University of North Carolina at Chapel Hill. The focus of my doctoral research was viral immunology and vaccines, specifically focusing on alphaviral vectors as vaccine delivery systems. I received my doctorate in 1999 and then took a position as a Research Scientist at AlphaVax, Inc. I held this position until 2001, when I assumed my current position.

3. I have read U.S. 5,951,975 (Falo et al.), U.S. 6,468,982 (Weiner et al.), U.S. 5,843,723 (Dubensky et al.), WO 95/32733 (Johnston 1), and U.S. 5,792,462 (Johnston 2), which have been cited by the Examiner in connection with the '837 application.

4. The Falo et al. patent (U.S. 5,951,975) is focused on prophylactic or therapeutic vaccination against tumors using an artificial cancer antigen to "cross prime" the immune response against tumor antigens. Falo's approach is based on the premise that immunization regimens with natural tumor antigens (*i.e.*, those taken from killed tumor cells, killed virus-infected tumor cells, or component proteins from tumor cells) do not elicit anti-tumor responses of sufficient potency or breadth to afford an anti-tumor effect. Falo's strategy proposes that an effective anti-tumor response can only be achieved by the co-delivery of an artificial antigen with a natural tumor antigen repertoire. Such "cross-priming" will enhance the immune response sufficiently to achieve recognition of additional tumor antigens. It is a fundamental assumption of the artificial antigen approach described in the Falo patent that direct immunization with a tumor antigen alone will not result in an effective anti-tumor response.

5. The Falo approach is completely distinct from the invention of the '837 application in at least two critical aspects. First, the '837 application does not claim vectors, compositions or methods for delivering an artificial antigen at all. The claims of the '837 application are concerned with alphavirus vector compositions encoding tumor antigens or slightly altered forms of tumor antigens that are antigenically similar to the tumor antigen. The claimed compositions of the '837 application are, in fact, contradictory to the Falo patent since Falo's invention was based on the premise that no delivery system vaccinating with tumor antigens, or slightly modified tumor antigens, alone would work, and that a cross-priming step with an artificial antigen is required to mediate any anti-tumor function. Second, the Falo approach requires the removal of the tumor from the patient, transfection of the artificial antigen into the tumor cells and the return of the modified cells to the patient; a so-called "*ex vivo*" cancer therapy. In contrast, the '837 application describes alphavirus-based vaccines, which can be directly administered to the patient. The claimed vaccine compositions do not require *ex vivo* manipulation of the tumor cells or the return of any component or population of these cells to the patient. Thus, the alphavirus vaccine compositions of the '837 application are different from, and

improved as compared with, the artificial cancer antigen vectors described by Falo in that the alphavirus vaccine compositions claimed in the '837 application can be used to deliver a cancer antigen expressed by the target cancer cell (*i.e.*, without the need for an artificial cancer antigen) and can be delivered both by *ex* vivo and direct *in vivo* administration, while the Falo methods are restricted to delivery of artificial cancer antigens by *ex vivo* manipulation of cells.

6. Because of the fundamental differences between the Falo artificial antigen approach and the invention in the '837 application, the anti-cancer effects described in the Falo patent cannot be extrapolated or applied to the alphaviral vector-based vaccine compositions of the '837 application. The fact that Falo demonstrated some success with one method of producing an anti-tumor response based on cross priming with an artificial antigen does not have any relevance to a completely distinct method of treating cancer by immunization with an alphavirus vector vaccine encoding a cancer antigen (or modified form that is antigenically similar) as described in the '837 application. In fact, as discussed above, Falo presumes that the direct immunization approach of the '837 application will not produce a sufficient anti-cancer effect, which is why Falo uses a more complex methodology that relies on cross-priming with an artificial antigen.

7. The Weiner patent (U.S. 6,468,982) is focused on genetic immunization using naked nucleic acid vaccines for applications including infectious disease, cancer and autoimmunity; *i.e.*, Weiner specifically states that "these genetic constructs are not incorporated within viral particles (Col. 12, lines 54-65). In the Background section of the patent, Weiner et al. go to great lengths to highlight their perceptions of the problems and inadequacies of other vaccine approaches as compared with their own approach of vaccination by direct injection of nucleic acid molecules. For example, in Col. 2, line 65, Weiner states: "Each of these vaccines carry severe drawbacks which render them less than optimally desirable for immunizing individuals against a particular pathogen." With particular respect to viral vectors, the Weiner patent gives multiple reasons in Col. 3, lines 30-50, as to why

viral vaccine vectors are undesirable, including the finding that "[i]n vaccines in which the pathogen genes are inserted into infective non-virulent vectors, many problems exist related to the immune response elicited against the vector antigens." Their point is that immune responses elicited against the vector are to the detriment of responses to the foreign antigen. For example, an immune response raised against the virus itself rather than the heterologous antigen of interest will result in virus clearance by the immune system and failure to elicit the desired immune response. In addition, Weiner states that the virus vectors can only be used one time as host immunity elicited against the vector will interfere with subsequent "booster" immunizations. It is clear that the intent of the Weiner patent is to provide an alternate method for vaccine delivery of antigens. Weiner et al. expend significant effort to differentiate their direct nucleic acid immunization strategy and to demonstrate how it is distinct from other vaccine mechanisms, in particular, viral vectors.

8. The Weiner method would be carried out with a "naked" DNA molecule, such as a plasmid, that encodes the foreign antigen. The system is dependent on the use of liposomes and a transfection agent to be co-administered with the nucleic acid, resulting in non-specific uptake by cells. In one embodiment, for example, the Weiner patent claims the use of bupivacaine as a transfection agent. After non-specific uptake of nucleic acid, the transfected cells must express the antigen at a sufficient level to elicit a host immune response.

9. In stark contrast, the vaccine compositions claimed in the '837 application are non-propagating, virus-like particles containing amplifiable RNA (referred to as a "replicon") expressing the tumor antigen (*i.e.*, "alphavirus replicon particles"). The alphavirus replicon particles are taken up by specific cellular receptors, present on only a subset of cells, which interact with alphavirus glycoproteins on the outer surface of the replicon particles. The replicon encoding and expressing the tumor antigen is replicated within the infected cell by virally encoded replicase enzymes, but it cannot spread to other cells. Expression from the

replicon in the infected cells is detectable for up to 3-5 days following inoculation. In contrast, naked DNA molecules are transcriptionally active for several weeks and persist in cells for at least several months (Gurananthan, S. et al. 2000 Ann Rev. Immunol. 18:927-74; Klinman, D. 2003 "Revising the 'Points to Consider' Document on DNA Vaccines," presentation by CBER/FDA available on-line at http://www.iabs.org/files2/Klinman.pdf; copies enclosed). Thus, the antigen expressed by the vaccine compositions described in the '837 application is amplified and expressed from the replicon at a high level within the cell over a relatively short time period to trigger an immune response. Alphavirus replicon particles are currently believed to be so highly effective as vaccine vectors because of the combination of specific infection of antigen-presenting cells of the immune system and high levels of expression elicited by alphavirus replicon particles. Further, alphavirus vaccines activate both arms of the immune response. In contrast, the naked nucleic acid vaccines used by Weiner are taken up non-specifically by host cells in the vicinity of the vaccination site, are replicated at low levels over a prolonged period of time, are completely dependent on cellular enzymes for their replication, and are biased toward only one arm of the immune response.

10. Thus, the '837 application uses a completely different mode of vaccine delivery than Weiner et al., and one that Weiner et al. argues has severe drawbacks. For the reasons described in paragraph 7 above, Weiner et al. would have predicted that the alphavirus vector compositions and methods of the '837 application would not be successful. As demonstrated by the unexpected efficiency of the alphavirus vaccine compositions claimed in the '837 application, the predictions of the Weiner patent were incorrect with respect to this alphavirus-based technology.

11. The genetic immunization approach of Weiner et al., which relies on non-specific delivery of naked DNA molecules using a transfection agent, is based on a completely different mechanistic approach than the alphavirus replicon vector-based vaccine compositions and methods of the '837 application. The use of a viral vectored vaccine encoding an antigen is fundamentally different from the use of a

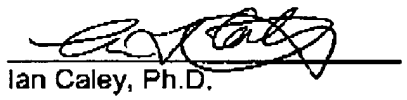
naked nucleic acid, which is not "vectored" at all, rather it is non-specifically taken up in the presence of a transfection agent. Thus, as discussed above for the Falo et al. patent, Weiner's results with nucleic acid vaccines are not relevant to the alphavirus based vector vaccine strategy of the '837 application.

12. An alphavirologist would not look to the Falo or Weiner patents for guidance in assessing the merits of an alphavirus based system for cancer vaccine delivery, and would not see the approaches described in these patents as relevant to an alphavirus vaccine delivery system. More particularly, an alphavirologist would not find the results reported by Falo and Weiner as useful in determining whether the pathogen vaccines of Johnston 1 or Dubensky could be modified for use in cancer therapy. In fact, at the time of filing of the '837 application, a general consensus in the vaccine field was that the immune responses elicited to DNA vaccines as described by Weiner were weak and biased toward only one and not both arms of the immune response.

13. Prior to the invention described in the '837 application, it would have been uncertain as to whether an alphavirus, in particular a non-propagating alphavirus replicon, could be used as a cancer vaccine and elicit an anti-cancer response. It was known that, in general, vaccine approaches to cancer treatment have low efficacy, which is likely attributable, at least in part, to host tolerance and the poor immunogenicity of cancer antigens. Further, as discussed by Weiner, viral vaccine approaches were known to have their own particular drawbacks, such as viral clearance by host immune responses against viral antigens, which prevents adequate expression of the cancer antigen and/or recognition of the antigen by the host immune system. In addition, the alphavirus replicon particles of the '837 application do not propagate (*i.e.*, spread) beyond the first population of infected cells. Thus, there is a relatively short timeframe for the replicon to amplify and express the cancer antigen in the first infected cells at sufficiently high levels to produce an immune response prior to toxicity and cell death as a result of the infection.

14. Thus, at the time of the invention in the '837 application, it could not have been reasonably expected that an alphavirus based vaccine, as described by Johnston 1 or Dubensky, expressing a cancer antigen could produce an effective anti-cancer effect. For the reasons discussed above, and as summarized in paragraph 12, the Falo and Weiner patents contributed nothing to reduce this uncertainty. The approaches and delivery systems described by Falo and Weiner are so different from the alphavirus vectored compositions claimed in the '837 application that an alphavirologist would not have looked to these documents for any guidance to evaluate the prospects for success for an alphavirus replicon vector-based vaccine.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Ian Caley, Ph.D.

December 11th, 2003
Date

Attachments:
Gurananthan et al.
Klinman et al.